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Oxygen Radical Absorbing Capacity of Phenolics in
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The antioxidant activity of phenolics in fruits of blueberry (*Vaccinium corymbosum* cv. Sierra), cranberry (*Vaccinium macrocarpon* cv. Ben Lear), wild chokeberry (*Aronia melanocarpa*), and lingonberry (*Vaccinium vitis-idaea* cv. Amberland) was determined in this study. The phenolic constituents and contents among the different berries varied considerably. Anthocyanins were found to be the main components in all these berries. Chlorogenic acid in blueberry, quercetin glycosides in cranberry and lingonberry, and caffeic acid and its derivative in chokeberry were also present in relatively high concentrations. Chlorogenic acid, peonidin 3-galactoside, cyanidin 3-galactoside, and cyanidin 3-galactoside were the most important antioxidants in blueberry, cranberry, wild chokeberry, and lingonberry, respectively. The contribution of individual phenolics to the total antioxidant capacity was generally dependent on their structure and content in the berries. Phenolics such as quercetin and cyanidin, with 3',4'-dihydroxy substituents in the B ring and conjugation between the A and B rings, had highly effective radical scavenging structures in blueberries, cranberries, chokeberries, and lingonberries. Phenolic acids such as caffeic acid also showed high antioxidant activity, probably due to its dihydroxylation in the 3,4 positions as hydrogen donors.

KEYWORDS: Antioxidant; anthocyanins; flavonoid; flavonol; phenolics; *Vaccinium*; *Aronia*

INTRODUCTION

Berries are rich in phenolic compounds as well as many essential nutritional components, such as flavonoids and phenolic acids, which exhibit a wide range of biological effects, including antioxidant (1–3) and anticarcinogenic properties (4). Epidemiological evidence suggests that high consumption of flavonoids may provide protection against coronary heart disease (5, 6), stroke (7), and lung cancer (8). A high scavenging activity of berry extracts toward chemically generated active oxygen species has been described in several studies (3, 9–13). In an earlier study, phenolic compounds from berry extracts inhibited human low-density lipoprotein (LDL) and liposome oxidation (11). Thus, high fruit consumption can significantly reduce the incidence and mortality rates of cancer, cardiovascular disorders, and other degenerative diseases caused by oxidative stress (14).

Flavonoids are polyphenolic compounds which contain a C₁₅ (C₆–C₃–C₆) basic skeleton and represent a large group of secondary plant metabolites (15). The diversity and complexity of the flavonoids found in berries depends on at least two factors: (i) the variety of aglycones and the high number of glycosides, sometimes in acylated form, and (ii) the possibility of condensation into complex molecules. Previous research has determined the antioxidant activity of some flavonoid com-

pounds (12, 16, 17) and attempted to define the structural characteristics which contribute to their activity (17, 18). Phenolic acids present in berries are hydroxylated derivatives of benzoic acid and cinnamic acid (15). Caffeic, chlorogenic, ferulic, sinapic, and *p*-coumaric acids appear to be more active antioxidants than hydroxy derivatives of benzoic acid, such as *p*-hydroxybenzoic, vanillic, and syringic acids (19).

Although intensive studies on phenolic constituents and structural analysis have been conducted in berries, the composition data are still incomplete (15, 20, 21). Furthermore, hydrolysis of glycoside bonds is often used in the extraction procedure in order to simplify the identification process; however, essential information of the authentic structure of phenolics is lost. It is well known that the degree of glycosylation significantly affects the antioxidant capacity of compounds (1). In addition, even though there is a wealth of data on the importance of antioxidants in conferring protection from oxidation, the correlation between antioxidant activity and chemical structure is far from clear. Different methods of assessment, varying substrate systems, and differential concentrations of active antioxidants have confounded these issues.

The purposes of this study were to (1) determine the antioxidant activity of phenolic compounds in blueberries, cranberries, chokeberries, and lingonberries in order to quantify the contribution of each phenolic to total antioxidant capacity and (2) investigate the activity–structure relationships of

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flavonoids and phenolic acids using the oxygen radical absorbance capacity assay (ORAC) of these four berry crops.

MATERIALS AND METHODS

Chemicals. Kaempferol, (*R*)-phycoerythrin (R-PE) from *Prophydium cruentum*, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO). 2',2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc. (Richmond VA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Aldrich (Milwaukee, WI). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). All anthocyanins and their aglycons were obtained from Indofine Chemical Co., Inc. (Somerville, NJ). Other authentic standards were obtained from Sigma and Fisher Scientific (Pittsburgh, PA).

Sample Preparation. Blueberries (cv. Sierra) used in this study were grown at the Henry A. Wallace Research Center, U.S. Department of Agriculture, Beltsville, MD. Cranberry (cv. Ben Lear) fruits were obtained from the Rutgers Blueberry and Cranberry Research Center, Chatsworth, NJ. Wild chokeberries were from Jonesboro, ME. Lingonberry (cv. Amberland) were obtained from St. John's, Newfoundland, Canada. All berries were harvested at the commercially ripe stage. Undamaged berries were selected, the seeds were removed (for cranberry only), and 30–40 berries were cut into small slices, mixed, and stored at -80°C until analyzed. To prepare the fruit extracts, 3–5-g samples of berries from four replicates of each berry crop were extracted twice with 10 mL of 80% acetone containing 0.2% formic acid using a Polytron (Brinkmann Instruments, Inc., Westbury, NY) for 2 min and then centrifuged at 20 000g for 20 min. The supernatants were combined and transferred to vials, stored at -80°C , and then used for analyses of ORAC, total phenolics, and total anthocyanins.

Total Phenolic and Anthocyanin Analysis. Total phenolic contents in berry extracts were determined according to the Folin–Ciocalteu procedure (22), and results are expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight. Total anthocyanin contents of berry extracts were measured using the pH differential method (23). Results are expressed as milligrams of cyanidin 3-glucoside (C 3-G) equivalents per gram of fresh weight.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assays for all samples were carried out according to procedures previously described by Wang and Lin (2), which were modified from a method described by Cao et al. (24). This assay measures the ability of antioxidant compounds in test materials to inhibit the decline of R-PE fluorescence that is induced by a peroxy radical generator, AAPH. The final results (ORAC value) were calculated using the differences of areas under the quenching curves of R-PE between a blank and a sample and are expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

HPLC Analysis of Berry Anthocyanins and Phenolic Compounds. High-performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in berry tissue samples. The supernatants (18 mL) from the extractions described above were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35°C , dissolved in 4 mL of acidified water (3% formic acid), and then passed through a C₁₈ Sep-Pak cartridge (Waters), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column, while sugars, acids, and other water-soluble compounds were eluted with 10 mL of 3% formic acid. Anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanol extract was passed through a 0.45- μm membrane filter (Millipore, MSI, Westboro, MA), and 20 μL was analyzed by HPLC. The samples were determined using a Waters Corp. (Milford, MA) HPLC system coupled with a photodiode array detector (Waters 990 Series) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20°C) into a reversed-phase NOVA-PAK C₁₈ column (150 \times 3.9 mm, particle size 4 μm) with a guard column (NOVA-PAK C₁₈, 20 \times 3.9 mm, particle size 4 μm) (Waters). The mobile phase consisted of 5% aqueous formic acid

Table 1. Antioxidant Activity (ORAC), Anthocyanin Content, and Phenolic Content in Fruit of Blueberry, Cranberry, Lingonberry, and Chokeberry

species	ORAC ^a (μmol of TE/g)	anthocyanin ^b (mg/g)	total phenolic ^c (mg/g)
blueberry (cv. Serra)	28.9b	1.20c	4.12b
cranberry (cv. Ben Lear)	18.5a	0.32a	3.15a
lingonberry (cv. Amberland)	38.1c	0.45b	6.52c
chokeberry (wild)	160.2d	4.28d	25.56d

^a Data expressed as micromoles of Trolox equivalents per gram of fresh weight.

^b Data expressed as milligrams of cyanidin 3-glucoside equivalents per gram of fresh weight. ^c Data expressed as milligrams of gallic acid equivalents per gram of fresh weight. Values within a column followed by different letters are significant at $p \leq 0.05$.

(A) and HPLC grade acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0–1 min, 4%; 1–10 min, 4–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–42 min, 20–45% B; 42–45 min, 45–100% B; 45–50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode array detector, and by chromatographic comparison with authentic markers (20, 21, 25–29). Individual flavonols and anthocyanins were quantified by comparison with an external standard of myricetin, quercetin, kaempferol, and cyanidin 3-glucoside. Scanning between 250 and 550 nm was performed, and data were collected by using the Waters 990 3D chromatography data system.

To assess the contribution of different individual phenolic compositions to antioxidant activity in berry extracts, the fraction containing each chromatographic peak was collected after separation by HPLC. Due to the interference of mobile-phase solvents in HPLC analysis on the ORAC assay, each peak fraction was concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35°C . The concentrated samples were dissolved in phosphate buffer (75 mM, pH 7.0) for ORAC analyses.

Statistical Analysis. Correlation and regression analyses of ORAC activity (*Y*) versus the total phenolics content (*X*) were carried out using NCSS (30). Data were subjected to analysis of variance using NCSS (30), and values of flavonoids and phenolic acids and their antioxidant capacities in berries were evaluated by the Tukey–Kramer multiple comparison test used in NCSS. Differences at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Antioxidant Activity of Flavonoids and Phenolics in Berries. The total anthocyanin, total phenolic content, and total antioxidant capacity (expressed as an ORAC value) in fruits of cranberry, blueberry, lingonberry, and chokeberry are shown in Table 1. On the basis of fresh weight of fruit, the chokeberry had significantly higher anthocyanin, phenolic content, and ORAC values than the three other berry crops. In this study, the total anthocyanin, phenolic content, and ORAC values in chokeberries were 4.28 mg of C 3-G/g, 25.56 mg of GAE/g, and 160.2 μmol of TE/g, respectively. Compared to blueberry and lingonberry, cranberry had generally lower anthocyanin content (0.32 mg of C 3-G/g fresh wt), total phenolic content (3.15 mg of GRE/g fresh wt), and total antioxidant capacity (18.5 μmol of TE/g fresh wt). Previous research showed that a linear relationship existed between total phenolic or anthocyanin content and ORAC in various berry crops (2, 31) and herbs (32). In this study, the correlation coefficient for ORAC (*y*) vs anthocyanins (*x*) was 0.951 ($y = 0.349x + 6.676$), and that for ORAC (*y*) vs total phenolics (*x*) was 0.998 ($y = 6.245x - 0.222$). In general, the correlation coefficient for antioxidant activity and phenolic content is better than that for ORAC value and anthocyanin. Prior et al. (33) reported that the correlation

Table 2. Concentration of Individual Phenolic Compounds in Blueberries, Cranberries, Chokeberries, and Lingonberries^a

compound	blueberry (cv. Sierra)		cranberry (cv. Ben Lear)		chokeberry (wild)		lingonberry (cv. Amberland)	
	concn ($\mu\text{g/g}$)	%	concn ($\mu\text{g/g}$)	%	concn ($\mu\text{g/g}$)	%	concn ($\mu\text{g/g}$)	%
chlorogenic acid	645.9 \pm 9.3	29.2						
vanillic acid			49.3 \pm 2.8	6.3				
caffeic acid derivative					1206.1 \pm 12.4	19.0		
caffeic acid			42.5 \pm 1.7	5.4	1411.4 \pm 14.3	22.2	63.4 \pm 3.6	6.4
<i>p</i> -coumaric acid							61.6 \pm 2.7	6.2
myricetin 3-arabinoside ^b	86.3 \pm 2.7	3.9	37.5 \pm 3.1	4.8				
quercetin 3-galactoside ^c	106.4 \pm 5.4	4.8	70.4 \pm 2.5	9.0	302.4 \pm 6.4	4.7	86.1 \pm 3.2	8.7
quercetin 3-glucoside	29.2 \pm 2.6	1.3			273.1 \pm 5.7	4.2		
quercetin 3-arabinoside	49.4 \pm 2.8	2.2	34.4 \pm 2.6	4.4			29.9 \pm 2.1	3.0
quercetin derivative	63.7 \pm 4.2	2.9					48.7 \pm 4.5	4.9
quercetin 3-rhamnoside			41.6 \pm 3.5	5.3			82.3 \pm 6.2	8.3
kaempferol 3-glucoside ^d	4.0 \pm 0.3	0.2	5.6 \pm 0.6	0.7			7.9 \pm 0.8	0.8
kaempferol derivative	3.4 \pm 0.2	0.2	4.0 \pm 0.5	0.5				
kaempferol							5.3 \pm 0.4	0.5
delphinidin 3-galactoside ^e	187.4 \pm 8.5	8.5						
delphinidin 3-glucoside	112.3 \pm 5.7	5.1						
delphinidin 3-arabinoside	94.0 \pm 3.4	4.3						
cyanidin 3-arabinoside			48.0 \pm 4.3	6.1	1424.3 \pm 18.2	22.4	62.7 \pm 5.3	6.3
cyanidin 3-galactoside	74.9 \pm 4.2	3.4	88.9 \pm 5.2	11.4	1256.3 \pm 11.5	19.8	486.9 \pm 7.9	49.3
cyanidin 3-glucoside	30.4 \pm 2.5	1.4	7.4 \pm 0.5	0.9	16.9 \pm 2.5	0.3	14.2 \pm 1.6	1.4
cyanidin 3-xyloside					469.0 \pm 8.6	7.4		
petunidin 3-galactoside	144.3 \pm 6.5	6.5						
petunidin 3-glucoside	70.3 \pm 4.2	3.2						
petunidin 3-arabinoside	128.9 \pm 5.2	5.8						
malvidin 3-galactoside	150.1 \pm 6.3	6.8						
malvidin 3-glucoside	114.6 \pm 4.2	5.2						
malvidin 3-arabinoside	114.5 \pm 3.6	5.2						
peonidin 3-galactoside			213.6 \pm 9.8	27.3				
peonidin 3-glucoside			40.4 \pm 2.8	5.2			41.3 \pm 2.8	4.2
peonidin 3-arabinoside			99.7 \pm 7.3	12.7				

^a Data expressed as mean \pm SEM. ^b Data of myricetin 3-arabinoside expressed as micrograms of myricetin equivalents per gram of fresh weight. ^c Data of quercetin aglycons expressed as micrograms of quercetin equivalents per gram of fresh weight. ^d Data of kaempferol aglycons expressed as micrograms of kaempferol equivalents per gram of fresh weight. ^e Data of anthocyanidin expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight.

coefficient for ORAC and total phenolic content was higher than that for ORAC and anthocyanin in fruit of *Vaccinium* species. These results suggest that the antioxidant activity of fruit is derived mainly from the contribution of phenolic compounds in fruits. However, little information is available on the contribution of individual phenolic compounds to total antioxidant activity in berry crops.

Solid-phase extraction (SPE) technology is usually used to purify samples in the preparation of fruit (34). Since berries contain large amounts of sugar, organic acids, pectin, and flavonoids, application of solid-phase extraction can crudely separate these compounds to obtain the desired extract. After SPE treatment (passing through the C₁₈ Sep-Pak cartridge), the ORAC values of cranberry, blueberry, lingonberry, and chokeberry were 16.6, 23.2, 21.2, and 138.2 μmol of TE/g fresh wt, respectively. These were lower than the ORAC values obtained without using SPE, which were 18.5, 28.9, 38.1, and 160.2 μmol of TE/g fresh wt for cranberry, blueberry, lingonberry, and chokeberry, respectively. Kähkönen et al. (3) reported the influence of sugar removal with Bond Elut C₁₈ SPE on the amount of phenolics in berry samples, and 5–11% reductions of total phenolics were found. This implies that the reduction in ORAC values after SPE treatment could be attributed in part to the loss of some water-soluble constituents of berry extracts (e.g., sugars, acids, ascorbic acid, glutathione, and other water-soluble compounds), which also possess antioxidant activity. Sugars and organic acids showed no antioxidant activity with the DPPD, FRAP, and TEAC methods (35, 36). However, citric, malic, and tartaric acids showed antioxidant activity when the DMPD method was tested (35). We also found no antioxidant activity of sugars and citric acid with the ORAC assay (data not shown). However, ascorbic acid and glutathione exhibited inhibition of peroxyl radical-induced PE oxidation in our ORAC

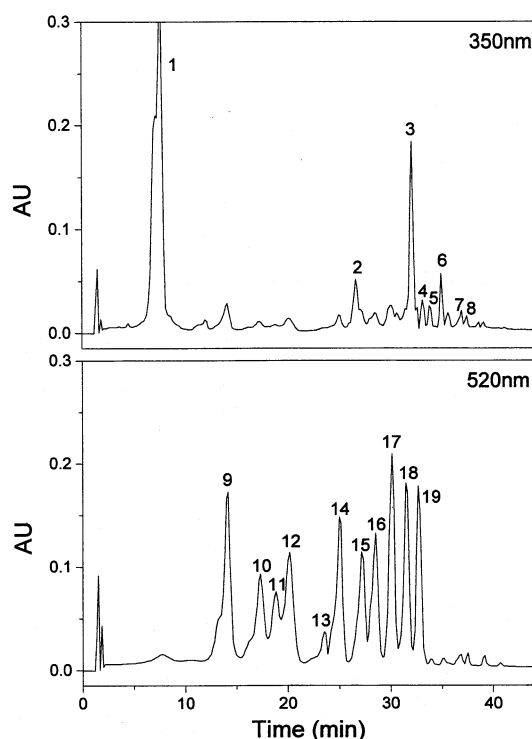


Figure 1. HPLC profile of blueberry phenolics: (1) chlorogenic acid; (2) myricetin 3-arabinoside; (3) quercetin 3-galactoside; (4) quercetin 3-glucoside; (5) quercetin 3-arabinoside; (6) quercetin derivative; (7) kaempferol 3-glucoside; (8) kaempferol derivative; (9) delphinidin 3-galactoside; (10) delphinidin 3-glucoside; (11) cyanidin 3-galactoside; (12) delphinidin 3-arabinoside; (13) cyanidin 3-glucoside; (14) petunidin 3-galactoside; (15) petunidin 3-glucoside; (16) petunidin 3-arabinoside; (17) malvidin 3-galactoside; (18) malvidin 3-glucoside; (19) malvidin 3-arabinoside.

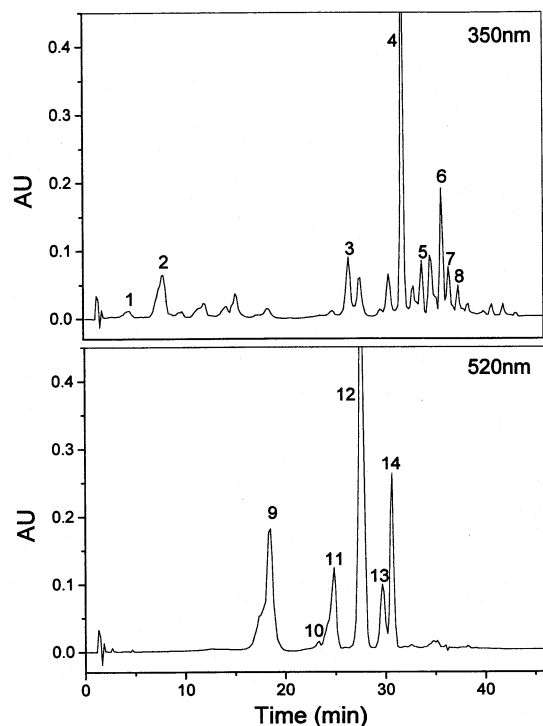


Figure 2. HPLC profile of cranberry phenolics: (1) vanillic acid; (2) caffeic acid; (3) myricetin 3-arabinoside; (4) quercetin 3-galactoside; (5) quercetin 3-arabinoside; (6) quercetin 3-rhamnoside; (7) kaempferol 3-glucoside; (8) kaempferol derivative; (9) cyanidin 3-galactoside; (10) cyanidin 3-glucoside; (11) cyanidin 3-arabinoside; (12) peonidin 3-galactoside; (13) peonidin 3-glucoside; (14) peonidin 3-arabinoside.

assay. The ORAC values for ascorbic acid and glutathione were 0.69 and 0.74, respectively. Cao and Prior (37) also showed ascorbic acid and glutathione antioxidant activity using the ORAC method.

Phenolics in berry extracts, separated and identified by using reversed-phase high-performance liquid chromatography (HPLC), are presented in **Table 2** and **Figures 1–4**. Considerable variation was found in phenolic compounds of different berries. Based on the total ORAC value of all identified compounds in each berry, the contribution of individual constituents to antioxidant activity is shown in **Table 3**. The ORAC value of chlorogenic acid of blueberry fruit was $4.76 \mu\text{mol}$ of TE/g fresh wt and accounted for 20.9%, which demonstrated that chlorogenic acid was the major contributor to antioxidant activity due to its high concentration in blueberry. Chlorogenic acid was a major cinnamic derivative found in large amounts in blueberries (26, 34) and has exhibited antioxidant activity, with a TEAC value of 1.24 (37). The total ORAC value of 11 anthocyanins identified in blueberry was up to $12.83 \mu\text{mol}$ of TE/g fresh wt and accounted for 56.3% of the total ORAC value in this study (**Table 3**). This indicated that anthocyanins showed significant contribution to antioxidant activity in blueberry, and therefore consumption of anthocyanin-rich fruit is beneficial to health. Among anthocyanin constituents of cranberry, peonidin 3-galactoside comprised 20.8% of the total ORAC value and had a high concentration ($1.91 \mu\text{mol}$ of TE/g fresh wt) as a main constituent in cranberry extract (**Table 3**). Cyanidin 3-galactoside was the most dominant anthocyanin, and ORAC values in chokeberry and lingonberry were 27.05 and $5.59 \mu\text{mol}$ of TE/g fresh wt, respectively (**Table 3**). In particular, 43.5% of antioxidant capacity was derived from cyanidin 3-galactoside in lingonberry. Andersen (28) identified cyanidin 3-galactoside as the main anthocyanin in Norwegian lingonberry with

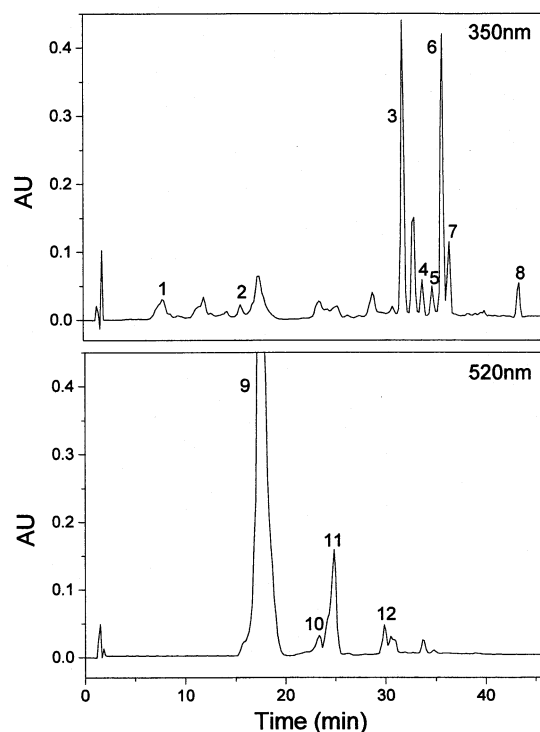


Figure 3. HPLC profile of lingonberry phenolics: (1) caffeic acid; (2) *p*-coumaric acid; (3) quercetin 3-galactoside; (4) quercetin 3-arabinoside; (5) quercetin derivative; (6) quercetin 3-rhamnoside; (7) kaempferol 3-glucoside; (8) kaempferol; (9) cyanidin 3-galactoside; (10) cyanidin 3-glucoside; (11) cyanidin 3-arabinoside; (12) peonidin 3-glucoside.

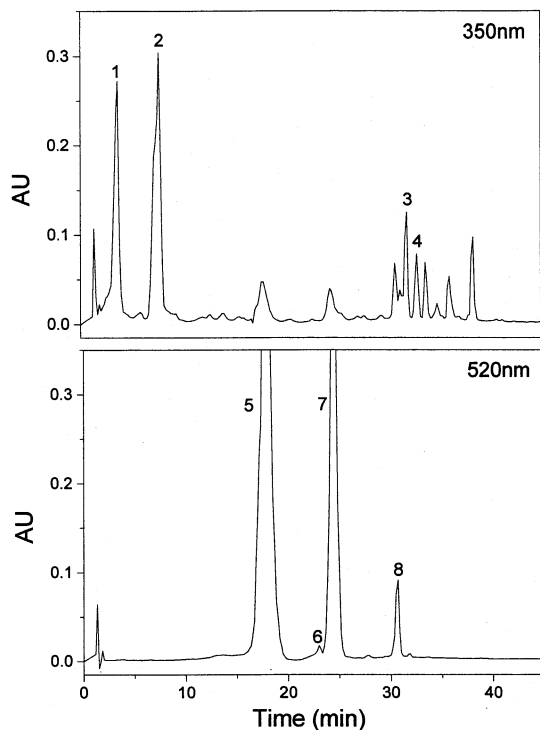


Figure 4. HPLC chromatograms of chokeberry phenolics: (1) caffeic acid derivative; (2) caffeic acid; (3) quercetin 3-galactoside; (4) quercetin 3-glucoside; (5) cyanidin 3-galactoside; (6) cyanidin 3-glucoside; (7) cyanidin 3-arabinoside; (8) cyanidin 3-xyloside.

relatively low amounts of the other anthocyanins. In addition to cyanidin 3-galactoside, cyanidin 3-arabinoside was also a major constituent in chokeberry and had a remarkably high

Table 3. Contribution of Identified Phenolics to the Antioxidant Activity in Blueberries, Cranberries, Chokeberries, and Lingonberries^{ab}

compound	blueberry (cv. Sierra)		cranberry (cv. Ben Lear)		chokeberry (wild)		lingonberry (cv. Amberland)	
	ORAC ($\mu\text{mol/g}$)	%	ORAC ($\mu\text{mol/g}$)	%	ORAC ($\mu\text{mol/g}$)	%	ORAC ($\mu\text{mol/g}$)	%
chlorogenic acid	4.76 \pm 0.61	20.9						
vanillic acid			0.40 \pm 0.02	4.4				
caffeic acid derivative					16.74 \pm 1.2	17.6		
caffeic acid			0.59 \pm 0.01	6.4	19.59 \pm 2.3	20.6	0.88 \pm 0.03	6.9
<i>p</i> -coumaric acid							0.53 \pm 0.02	4.1
myricetin 3-arabinoside	1.24 \pm 0.05	5.4	0.54 \pm 0.01	5.9				
quercetin 3-galactoside	1.42 \pm 0.02	6.2	0.94 \pm 0.03	10.2	4.04 \pm 0.4	4.2	1.15 \pm 0.09	9.0
quercetin 3-glucoside	0.46 \pm 0.01	2.0			4.31 \pm 0.5	4.5		
quercetin 3-arabinoside	0.66 \pm 0.02	2.9	0.46 \pm 0.02	5.0			0.40 \pm 0.02	3.1
quercetin derivative	0.95 \pm 0.04	4.2					0.72 \pm 0.06	5.6
quercetin 3-rhamnoside			0.61 \pm 0.02	6.6			1.26 \pm 0.09	9.8
kaempferol 3-glucoside	0.28 \pm 0.02	1.2	0.39 \pm 0.02	4.2			0.55 \pm 0.04	4.3
kaempferol derivative	0.21 \pm 0.01	0.9	0.25 \pm 0.01	2.7				
kaempferol							0.33 \pm 0.02	2.6
delphinidin 3-galactoside	2.08 \pm 0.07	9.2						
delphinidin 3-glucoside	1.42 \pm 0.05	6.2						
delphinidin 3-arabinoside	1.01 \pm 0.04	4.4						
cyanidin 3-arabinoside			0.59 \pm 0.02	6.4	17.49 \pm 2.8	18.4	0.77 \pm 0.08	6.0
cyanidin 3-galactoside	0.86 \pm 0.03	3.8	1.02 \pm 0.05	11.1	27.05 \pm 3.2	28.5	5.59 \pm 0.12	43.5
cyanidin 3-glucoside	0.45 \pm 0.01	2.0	0.11 \pm 0.01	1.2	0.25 \pm 0.0	0.3	0.21 \pm 0.02	1.6
cyanidin 3-xyloside					5.58 \pm 0.7	5.9		
petunidin 3-galactoside	1.33 \pm 0.08	5.8						
petunidin 3-glucoside	0.78 \pm 0.07	3.4						
petunidin 3-arabinoside	1.16 \pm 0.05	5.1						
malvidin 3-galactoside	1.43 \pm 0.04	6.3						
malvidin 3-glucoside	1.17 \pm 0.03	5.1						
malvidin 3-arabinoside	1.14 \pm 0.02	5.0						
peonidin 3-galactoside			1.91 \pm 0.07	20.8				
peonidin 3-glucoside			0.44 \pm 0.01	4.8			0.45 \pm 0.03	3.5
peonidin 3-arabinoside			0.95 \pm 0.02	10.3				
total	22.81	100	9.20	100	95.05	100	12.84	100

^a Data expressed as mean \pm SEM. ^b Data expressed as micromoles of Trolox equivalent per gram of fresh weight.

ORAC value (17.49 μmol of TE/g fresh wt). Caffeic acid and its derivative were found to be two major phenolic acids in chokeberry, and both had a high proportion of antioxidant activity, with 20.6% and 17.6%, respectively (Table 3).

Using ORAC values of all the identified constituents, total antioxidant capacities were summed and calculated for each berry. These summed total antioxidant capacities were 9.20, 12.84, 22.81, and 95.05 μmol of TE/g fresh wt for cranberry, lingonberry, blueberry, and chokeberry, respectively. Compared to their actual measured total antioxidant capacity (Table 1), the calculated values were found to be lower (Tables 1 and 3), and this may have been due mainly to unmeasured substances in the fruit extract or synergistic interactions between the measured components.

Structure–Antioxidant Activity Relationships of Berry-Derived Flavonoids and Phenolic Acids. To assess structure and antioxidant activity relationships in some flavonoids and phenolic acids of berries, the antioxidant capacity of every phenolic compound was calculated and expressed as micromoles of Trolox equivalents per milligram (Table 4). Compared to other anthocyanin aglycons, cyanidin showed higher antioxidant activity, and the order of antioxidant potency defined by ORAC values was cyanidin > delphinidin > malvidin \approx peonidin \approx petunidin. Previous reports demonstrated that the difference in antioxidant capacity could be ascribed to individual molecular structure. Some possible explanations are as follows: (i) increasing the number of hydroxyl groups may increase antioxidant activity (17); (ii) the *o*-dihydroxy structure in the B ring confers higher stability to the radical form and participates in electron delocalization (1), and thus, the dihydroxylation in the 3',4' positions of the B ring plays an important role in antioxidant activity; (iii) the glycosylation of flavonoids may reduce their activity when compared to that of the corresponding aglycons (38); (iv) the unsaturation in the C ring allows electron

Table 4. Hierarchy of Trolox Equivalent Antioxidant Activity of Selected Phenolics in Blueberries, Cranberries, Chokeberries, and Lingonberries

aglycon or compound	free OH substituents	glycosylation	AA ^a (μmol of TE/mg)
cyanidin	3,5,7,3',4'	3-galactoside	11.5 \pm 0.81
		3-arabinoside	12.3 \pm 0.98
		3-glucoside	14.8 \pm 1.10
		3-xyloside	11.9 \pm 0.78
delphinidin	3,5,7,3',4',5'	3-galactoside	11.1 \pm 0.51
		3-arabinoside	10.7 \pm 0.33
		3-glucoside	12.6 \pm 0.94
petunidin	3,5,7,4',5'	3-galactoside	9.2 \pm 0.83
		3-arabinoside	9.0 \pm 0.46
		3-glucoside	11.1 \pm 0.81
malvidin	3,5,7,4'	3-galactoside	9.8 \pm 1.23
		3-arabinoside	10.0 \pm 1.05
		3-glucoside	10.2 \pm 1.42
peonidin	3,5,7,4'	3-galactoside	8.9 \pm 0.93
		3-arabinoside	9.5 \pm 1.56
		3-glucoside	10.9 \pm 1.52
quercetin	3,5,7,3',4'	3-galactoside	13.4 \pm 1.44
		3-arabinoside	13.4 \pm 0.52
		3-glucoside	15.8 \pm 1.65
		3-rhamnoside	14.7 \pm 1.22
myricetin	3,5,7,3',4',5'	3-arabinoside	14.4 \pm 0.99
chlorogenic acid			7.4 \pm 0.87
caffeic acid			13.9 \pm 1.25
<i>p</i> -coumaric acid			8.6 \pm 0.54
vanillic acid			8.1 \pm 0.66

^a Data of antioxidant activity (AA) was expressed as micromoles of Trolox equivalent per milligram of compounds.

delocalization across the molecule for stabilization of aryloxy radicals due to existing conjugation (1); and (v) the 3- and 5-OH groups with 4-oxo function in the A and C rings are required for maximum radical scavenging potential (1). By comparing anthocyanin aglycons (Figure 5), it was found that different hydroxyl (OH) and methoxy group substitutions influence the antioxidant activity of anthocyanins, even though they show the same structure in the A and C rings. Monophenols in the B

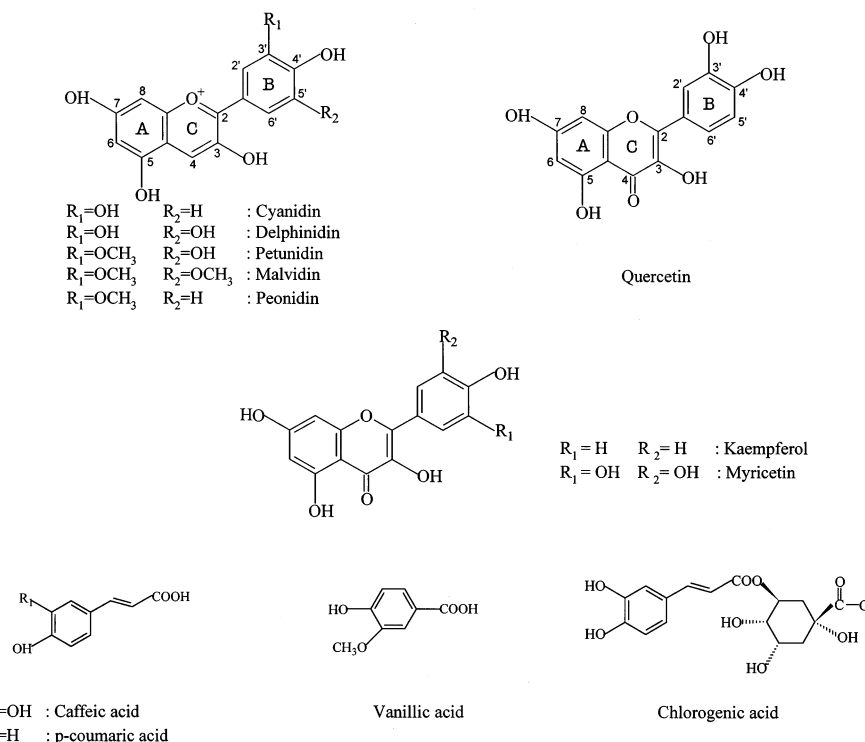


Figure 5. Structure of phenolics in cranberry, blueberry, lingonberry, and chokeberry.

ring, such as petunidin, malvidin, and peonidin, give lower ORAC values compared to those of compounds with 3',4'-OH substitution. Thus, insertion of a methoxy group in the 3' position of the B ring will decrease the antioxidant capacity. In addition, there are small influences of an additional methoxy or hydroxyl group in the 5' position, although more hydroxyl groups can increase the antioxidant activity. Moreover, the appearance of 5'-hydroxylation decreased ORAC values in the presence of 3',4'-OH as cyanidin vs delphinidin, and this was in agreement with previous results reported by Wang et al. (39).

Flavonols (quercetin, myricetin, and kaempferol) showed high antioxidant activity with a structure which allows it to have more effective antioxidant activity than that of anthocyanins (Figure 5 and Table 4). The 2,3 double bond in conjunction with a 4-oxo function in the C ring of quercetin allowed electron delocalization from the B ring, showed extensive resonance, and resulted in significant effectiveness for radical scavenging (1). Quercetin has a structure similar to that of cyanidin in the A and B rings (3',4'-dihydroxy substituents in the B ring and conjugation between the A and B rings) and the same number and arrangement of five hydroxyl groups. This suggested that quercetin may contribute significantly to antioxidant potential because its structure effectively satisfies the stabilization of the aryloxy radical after hydrogen donation. An additional OH group at B ring 5' position of quercetin, as in myricetin, increases the ORAC. Wang et al. (39) also reported that the antioxidant activity of myricetin was higher than that of quercetin in terms of ORAC value (4.32 vs 3.2). Kaempferol, with a structure related to that of quercetin, but with only a single 4'-OH group in the B ring, has just 27% of quercetin's antioxidant activity.

Glycosylation of the anthocyanidins and quercetin may modulate antioxidant activity (39). The preferred glycosylation site on the flavonoids is the 3 position and, less frequently, the 7 position. Glucose is the most common sugar residue, but others include galactose, rhamnose, and xylose (1). As Table 4 shows,

different sugars may have different antioxidant activity in the same aglycon. Using quercetin as an example, 3-glycosylation in the C ring for glucose had the highest antioxidant activity compared to the others, and the order of antioxidant potency usually was 3-glucoside > 3-rhamnoside > 3-arabinoside \approx 3-galactoside. The difference in antioxidant potency comes from the orientation, number, and distribution of hydroxyl group in sugars. This is in agreement with the results reported by Wang et al. (39), who indicated that the only difference between glucose and galactose of cyanidin is the orientation of one hydroxyl group at the pyran ring.

Caffeic acid, chlorogenic acid, *p*-coumaric acid, and vanillic acid are widely distributed in berry crops as natural antioxidants. Their antioxidant activities are associated to some extent with the number of hydroxyl groups in their molecular structure (1). For example, in comparing two hydroxycinnamic acids, caffeic acid and *p*-coumaric acid, the antioxidant activity value of the former (13.9 μmol of TE/mg) was higher than that of the latter (8.6 μmol of TE/mg) (Figure 5 and Table 4). It is likely that dihydroxylation in the 3,4 position could enhance the antioxidant potency by making more available hydrogen donors. Chlorogenic acid (7.4 μmol of TE/mg) showed lower antioxidant activity compared to that of caffeic acid (Table 4). Vanillic acid is a benzoic acid derivative and had values similar to those of *p*-coumaric acid. Substitution of the 3-hydroxyl group by a methoxy group would influence its antioxidant activity (1, 38).

In summary, blueberries, cranberries, chokeberries, and lingonberries contain large amounts of phenolics and also have high antioxidant activities. Considerable variation in antioxidant activity was found in various phenolic compounds of different berries. The contribution of individual phenolics to total antioxidant capacity was generally dependent on their structure and content in berries. The results of this study provide guidance as to the relative potential health benefits of different berries.

ABBREVIATION USED

AAPH, 2',2'-azobis(2-amidinopropane) dihydrochloride; DPPH, α,α -diphenyl- β -pyridylhydrazyl; DMPD, *N,N*-dimethyl-*p*-phenylenediamine; FRAP, ferric reducing ability of plasma; TEAC, Trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbance capacity; R-PE, (*R*)-phycoerythrin; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

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